

- TOOTY\* CHITON
- (j) filtering and washing the colourless TCB hapten with acetone and drying in air;
  - (k) using silica gel precoated aluminum plates and a mixture of chloroform and methanol 85:15 as eluent showed a single spot in TLC analysis Rf- 0.45 detected by spraying with 2% o-tolidine in acetone and exposure to Uv light/sunlight, at a melting range of 169-70 ° C.
  - (l) synthesizing the active ester of hapten 2,4,5-T- $\beta$ - alanine at melting range of 102-104 °C by dissolving in dichloromethane .
  - (m) adding N- hydroxysuccinimide and the mixture is cooled in an ice-bath;
  - (n) adding Dimethylsulphoxide(DMSO) dropwise to the mixture until the hapten is dissolved;
  - (o) adding Dicyclohexylcarbodiimide to the mixture followed by adding dimethylaminopyridine catalyst;
  - (p) stirring the mixture overnight and the temperature slowly raised to the room temperature;
  - (q) filtering and evaporating acetone; and
  - (r) separating the active ester as a colorless solid.

Yet another embodiment of the present invention, wherein harvesting of antibodies is conducted as follows:

- (a) obtaining egg yolk without rupturing the yolk;
- (b) adding 100 ml of Tris buffer saline for every 10 ml of yolk;
- (c) removing the precipitate by centrifugation;
- (d) adding to the supernatant the precipitating solution of magnesium chloride and phosphotungstic acid for centrifuging;
- (e) discarding the pellet;
- (f) adding to the supernatant a water soluble protein fraction 12% polyethylene glycol;
- (g) incubating for 10 minutes and then centrifuging again;
- (h) precipitating out the antibody;
- (i) adding 10 ml of 10mM phosphate buffer to dissolve the precipitate;
- (j) cooling the antibody solution 0°Cl;

- (k) adding 10 ml of pre-cooled ethanol;
- (l) centrifuging the solution at 4<sup>0</sup>C and dissolving the sediment in 10 mM phosphate buffer; and
- (m) dialyzing against phosphate buffer for 24 h at 4<sup>0</sup>C to obtain the yield of antibodies.

Still another embodiment of the present invention, wherein harvesting of antibodies can also be conducted as follows:

- (a) obtaining the egg yolk from the eggshell without rupturing the yolk membrane;
- (b) adding for every 10 ml of yolk, 10 ml of distilled water;
- (c) adding about 0.15 % of kappa- carragenanin and left to stir for 30 minutes at room temperature;
- (d) filtering and centrifuging the solution at for 15 minutes;
- (e) passing through the DEAE – sephacel column prepared with 20 mM phosphate buffer pH 8.0;
- (f) eluting with 0.2 M phosphate buffer pH 8.0;
- (g) collecting the eluate and the absorbance read at 280 nm; and
- (h) pooling and storing the peak fractions containing the antibody at 4<sup>0</sup> C.

Yet another embodiment of the present invention, wherein the lipid from egg yolk is precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride and centrifuged obtaining the antibody yield up to 75% from supernatant.

Still another embodiment of the present invention, wherein pH of the water soluble protein fraction obtained after the removal of the lipids is adjusted to pH 5.0 to further precipitate out the antibodies for obtaining a yield of 80 –90%.

Yet another embodiment of the present invention, wherein the yield of antibody is to the extent of 73%.

Still another embodiment of the present invention, wherein the hyper immune eggs are collected daily and stored 40°C until further use.

Yet another embodiment of the present invention, wherein commencing the production of the antibody from 7<sup>th</sup> day after the immunization and continued for 60 days.

Still another embodiment of the present invention, wherein the titer of the antibody produced is 165-225 mg/ml.

Yet another embodiment of the present invention, wherein production of the egg yolk antibody is more/ equally sensitive to the polyclonal / monoclonal antibodies produced using mammals.

Still another embodiment of the present invention, wherein production of the egg yolk antibodies relates to small molecules of pesticides.

Yet another embodiment of the present invention, wherein the production of egg yolk antibodies binding to the following organochlorine insecticides selected from:

- a) (DDT – 1,1' – (2,2,2 – Trichloroethylene ) bis ( 4 – chlorobenzene),
- b) HCH – 1,2,3,4,5,6 – hexachlorohexane
- c) ENDOSULPHAN – 6,7,8,9,10,10 – hexachloro – 1,5, 5a,6,9,9a, hexahydro – 6,9 – methano – 2,3,4 – benzodioxathiepin – 3 – oxide.)

The following examples are given by way of illustration of the present invention and therefore should not be construed to limit the scope of the present invention.

## EXAMPLES

### EXAMPLE –1

The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg